Enzymatic assay for the determination of acetic acid in foodstuff and other sample materials
2 x 50 ml R1 und 2 x 12.5 ml R2 (50 assays)

For *in vitro* use only
Store between +2 - +8 °C

Principle

Enzymatic test with Acetate kinase (AK), ADP-dependent Hexokinase (ADP-HKP) and Glucose-6-Phosphate Dehydrogenase (G6P-DH). NADH is produced and is measured at 340 nm:

Acetate + ATP —— AK ⟶ Acetyl phosphate + ADP

ADP + D-Glucose — ADP-HKP ⟶ D-Glucose-6-Phosphate + AMP

D-Glucose-6-Phosphate + NAD^+ — G6P-DH ⟶
6-Phosphoglucono-δ-lactone + NADH + H^+

Reagents

The reagents are ready-to-use.
Reagent 1: two vials ≥ 50 ml (Buffer, NAD)
Reagent 2: two vials ≥ 12.5 ml (AK, ADP-HKP, G6P-DH)
Calibrator-Set: 4 vials ≥ 3.5 ml each (0.02 - 1.3 g/l acetic acid)

The reagents are stable up to the end of the indicated month of expiry if stored at 2 - 8 °C, even after repeated opening (if not contaminated during handling). Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Use liquid and clear samples directly, or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Clarify samples containing proteins with Carrez clarification
- Crush and homogenize solid or semi-solid samples and extract with water (e.g. 30 min at 60 - 70°C). Filter or centrifuge, or apply Carrez clarification if necessary.
- For fat containing samples, extract with hot water, cool down to separate the fat (fridge or ice), remove the fatty layer and filter the aqueous part

Assay procedure

Wavelength: 340 nm
Optical path: 1 cm
Temperature: 20 - 25 °C
Measurement: Against air or against water
Sample solution: 0.02 bis 1.3 g/l

	Reagent blank (RB)	**Samples / Calibrators**
Sample / Calibrator	-	100 µl
Dist. water	100 µl	-
Reagent 1	2000 µl	2000 µl
Mix, incubate for 1 min at 37 °C or 3 min at 20 - 25 °C. Read absorbance A_1 in time, then add:		
Reagent 2	500 µl	500 µl
Mix, incubate 10 min at 37°C or 15 min at 20 - 25 °C. Read absorbance A_2 in time (no endpoint determination).		

The reagent blank must be performed once for each run and subtracted from each sample result.

Calculation of results

1. $\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
With df = dilution factor of optical densities:

df = (sample volume + R1) / (sample volume + R1 + R2) = 0.808

2. The calibration curve is determined in Excel using a 4th degree polynomial. The target concentration values of the calibrators are plotted against the corresponding Δ A values. The concentration of the samples is determined using the polynomial equation or directly from the graph. An Excel evaluation table is available on request.

Example with typical absorbance values:

	Acetic acid (g/l)	**A_2**	**Δ A (minus blank)**
Calibrator 1	0.02	0.288	0.077
Calibrator 2	0.1	0.534	0.323
Calibrator 3	0.3	0.940	0.729
Calibrator 4	1.3	1.871	1.660

3. Calculation for solid samples:

$$\text{content}_{analyte}\ [g/100\ g] = \frac{C_{analyte}\ [g/l]}{\text{weight}_{sample}\ [g/l]} \times 100$$

Test performance

Specificity

The determination is specific for acetic acid. Interferences were measured for ascorbic acid up to 1.0 g/l, for citric acid up to 2.5 g/l and for tartaric acid up to 3.5 g/l and can be excluded. Interferences were measured for glycerol up to 25 g/l and for sulphite (SO_2) up to 1 g/l and can be excluded.

Measuring range

The recommended measuring range is 0.02 to 1.3 g/l, in order to ensure Δ A ≅ 1.5 (A). If this range is exceeded, the samples should be diluted with distilled water to a concentration within the measuring range. The dilution factor must be included in the calculation.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

- LoD = 2.5 mg/l
- LoQ = 4.5 mg/l

Calibration and Automation

The calibration stability is 7 days.
Application sheets for automated systems are available on request.

Disclaimer

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